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(54) Title: FUNGICIDAL CARBOHYDRATE PREPARATIONS

(57) Abstract

The present invention relates to the use of saccharides as enhancers of fungicidal agents, compositions comprising saccharides in combination with fungicidal agents, and methods for combating pathogens by applying such compositions.

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Fungicidal Carbohydrate Preparations

FIELD OF THE INVENTION

The present invention relates to the use of saccharides as enhancers of biocidal agents, compositions comprising said saccharides, and methods for combating pests by applying said compositions.

BACKGROUND OF THE INVENTION

The primary methods of controlling pests on crop plants comprises treatment with synthetic chemical biocides. However, the exposure of man and habitats to increasing amounts of biocides has come under criticism, resulting in search for environmentally safer methods, including the use of combinations of biocides to reduce the amounts of application.

It is well known that enzymes may enhance the biocidal effect of synthetic chemical biocides in a synergistic way. This includes, enzymes such as proteases, esterases, cellulases, hemicellulases, lipases, pectinases, and endo chitinases.

Poulose, A.J., in Koeller, W., ed., Target Sites of Fungicide Action, CRC Press, Boca Raton, Florida, 1992, at pages 313-314 reviews number of disclosures directed to synergistic interaction of different lytic enzymes produced by a variety of microorganisms with a small number of antifungal compounds including amphotericin B, benomyl, polyoxin B, kitazin P and nikkomycin.

EP 197622 (Kao Corporation), published 15.10 1986 discloses the enhancement of biocides against plants or insects by applying an esterase.

EP 272002 (Genencor Inc), published 22.06 1988 discloses increased pharmacological effect of agricultural chemicals by applying cell wall degrading enzymes. The disclosed enzymes is a mixture of cellulase, hemicellulase, lipase, peptidase and pectinase activity.

WO 94/13784 (Cornell University), published 23.06.1994 discloses the antifungal synergistic combination of an enzyme fungicide and a non-enzymatic fungicide and the use thereof. The disclosed enzymes are glucanolytic enzymes and cellulases (EC

3.2.1.4) from *Trichoderma harzianum*. The disclosed non-enzymatic fungicides are selected from the group consisting of sterol synthesis inhibiting fungicides (e.g. flusilazole and miconazole) and thiol group inactivating fungicides.

WO 94/24271 (Cornell Res. Fdn. Inc), published 27.06.1994 further discloses inhibition of germination, replication or growth by use of chitin and b-1,3-glucan containing fungus. The antifungal composition contains endo chitinase, chitin-1,4-b-chitobiosidase, N-acetyl-b-glucosidase isolated from *Trichoderma harzianum* strain P1.

US 5395530 (Nalco Chemical Co.), published 07.03.1995 discloses inhibition of filamentous microorganism growth by addition of a disaccharide enzyme and a biocide.

Investigations regarding the composition of commercial formulations of enzyme preparations in combination with biocides have not been conducted yet, typically comprise such formulations saccharides, e.g. sorbitol.

Use of saccharides as stabilising agents of proteins, especially enzymes, is well known. DE 2038103 (Henkel and Cie) published august 1972 discloses the use of monosaccharide and disaccharide alcohols as enzyme stabilising agents in dishwashing compositions. JP 760903 (Meito Sangyo) published 03.09.1976 discloses monosaccharides and oligosaccharides as enzyme stabilisers.

Saccharides as growth enhancer of plants is disclosed in JP 1117807 (Mitsui & Co) published 10.05.1987. Here, glucose and fructose is sprayed directly on the leaves of plants with a growth promoting effect. No biocidal effect is disclosed.

Holmstrup, Martin (1995). Comp. Biochem., vol. 111A, # 2 pp 255, further discloses the polyol (e.g. sorbitol) accumulation in earthworm cocoons induced by dehydration.

Saccharide related compounds used as biocides is only described in DE 3545908 (Henkel) published 25.06.1987. The application discloses the use of a generic alkyl glycoside compound as pesticide alone or as enhancer in combination with known biocides. This invention does however not disclose the use of free saccharides as enhancers of biocidal agents.

SUMMARY OF THE INVENTION

The invention relates to a fungicidal composition comprising a saccharide in combination with a biocidally active preparation in admixture with a fungicidally acceptable diluent or carrier.

The composition according to the invention may further comprise an enzymatic composition, being either an enzyme, an enzyme preparation, or an enzyme complex.

The invention furthermore relates to the use of saccharides for enhancing the fungicidal effect of a fungicide.

The invention also relates to a method of controlling fungi by application of a composition of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Visualises the evaluation system ranking plants from 0 to 9 depending on the damage from the pest. The illustrated pest is Phytopthora infestans on potato plants.

Figure 2: Illustrates four tomato plants inoculated with Phytopthora infestans: From left to right. Number one is treated with 100 ppm Mancozeb and 1% Celluclast®, number two is treated with 100 ppm Mancozeb, the third is untreated, and the fourth is treated with 1 % Celluclast®.

DETAILED DESCRIPTION OF THE INVENTION

From the publications summarised above it appears that lytic enzymes have been described in combination with biocides containing no enzymes to be of use in plant protection when applied in combination with biocides as biocontrol agents.

We have however discovered, that an enhancing activity of biocides in combination with enzyme formulations (e.g. Celluclast®, was maintained after autoclavation. In order to examine this phenomenon, tests were conducted with the additives in the Celluclast formulation: Saccharides and salts.

The present invention discloses for the first time the use of saccharides as enhancers of biocides to obtain improved pest control.

More particularly the present invention comprises a composition of a saccharide in combination with a biocidally active preparation in admixture with a biologically acceptable diluent or carrier.

Said saccharide comprises monosaccharides, disaccharides, polyols, or polypeptides, and salt or acids of said groups. Said monosaccharides comprises ribose, arabinose, xylose, glucose, fructose, mannose, or galactose, said disaccharides comprises sucrose, lactose, maltose, or cellobiose, said polyols comprises sorbitol, mannitol, galactitol, ribitol, allitol, or xylitol, said polysaccharides comprises cellulose, pectin, algin, inulin, amylose, or dextrose and said acids comprises gluconic acid, mannonic acid, or arabinonic acid.

The concentration of the saccharide in the composition of the present invention as applied to plants is preferably within the range of 0.001 to 70 vol.%.

It may be 0.001%, 0.01%, 0.05%, 0.1%, 0.3%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 9.5%, 10.5%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69% or 70%.

In a primary composition the amount of saccharide can vary widely and be, for example, from 5 to 90 percent of the volume.

It was furthermore demonstrated that a facilitating effect of the interaction between a lytic enzyme complex and a biocidally active compound or composition is often obtained by adding a saccharide. This facilitating effect allows application of both enzyme and the other biocide in considerably lower dosages than the ones usually applied, while obtaining the same or improved control effect of the pest.

The enzymatic composition comprises either an enzyme, an enzyme preparation, or an enzyme complex. Said enzymatic

composition may further comprise a mixture of enzymes or enzyme activities.

In a preferred embodiment of the invention said enzyme preparation comprises one or more enzymes selected from the group comprising cellulases or hemicellulases. Said enzyme preparation further being a microbial, e.g. bacterial or fungal origin. In a preferred embodiment of the invention said enzyme comprises Celluclast® or other cellulytic compositions containing saccharides.. In another preferred embodiment of the invention said enzyme preparation comprises enzymes that are substantially composed of one enzyme activity (monocomponent enzymes). In a preferred embodiment of the invention said monocomponent enzyme comprises Xylanase I, Xylanase II and Endoglucanase III.

The concentration of said enzyme lies in the range from 0.001 to 5 w/w, preferably in the range from 0.01 to 2% w/w, more preferably in the range from 0.05 to 1.5%w/w, even more preferably between 0.1 to 1.0%w/w.

The invention is applicable to fungi, and the invention thus also provides a method of combating fungi at a locus infested or liable to be infested therewith, which comprises applying to the locus a fungicidally active preparation of the invention, and in a preferred embodiment of the invention said locus is subjected sequentially or in a mixture to the saccharide preparation of this invention as well as said other biocide.

The invention provides a composition comprising a selected saccharide and a fungicide in admixture with an acceptable diluent or carrier.

Depending on the circumstances, the environmental conditions or other factors, a composition of the invention in addition to said fungicidally active compounds may also contain other active ingredients, e.g. pesticides, such as further fungicides, herbicides, nematocides, rodenticides, bactericides, molluscicides or acaricides, or plant nutrients or fertilisers.

Examples of synthetic fungicides which can be combined with the saccharide preparation of the invention either alone or in combination with an enzyme include the acetamides, examples of which are the common names cymoxanil and ofurace; the acylalanines, examples of which are the common names benalaxyl

and metalaxyl; the amines, of which examples are the common names 2-aminobutane and diphenylamine; the anilides, examples of which are the common names benodanil and flutalonil; the antibiotics, of which examples are the common names blasticidin-S and polyoxins; the benzimidazoles, of which examples are the common names benomyl and thiabendazole; the carbamates, of which examples are the common names diethofencarb and propamocarb; the conazoles, of which examples are the common names prochloraz and propiconazole; the dicarboximides, of which examples are the common names iprodione and vinclozolin; the dinitrophenyl derivatives, of which examples are the common names dinocap and DNOC; the dithiocarbamates, examples of which include the common names mancozeb and maneb; the dithiolanes, of which examples are the common names chinomethionat and isoprothiolane; the guanidines, examples of which are the common names dodine and iminoctadine; the morpholines, of which examples are the common names dimetomorph and tridemorph; the inorganics, of which examples are the common names Bordeaux mistrue and copper sulfate; the isoxazoles, of which examples are the common names drazoxolon and hymexazol; the organochlorine derivatives, of which examples are the common names anilazine and hexachlorbenzene; the organocopper derivatives, of which examples are the common names copper naphthenate and oxine copper; the organophosphorous derivatives, of which examples are the common names fosetyl and pyrazophos; the organotins, of which examples are the common names fentin acetate and fentin hydroxide; the oxazolidines, of which examples are the common names chlocolinate and oxadixyl; the phenylamides, examples of which are the common names fenfuram and mepronil; the phenylpyrroles, of which examples are the common names CGA 173506 and fenpiclonil; the piperidines, of which examples are the common names fenpropidin and piperalin; the phtalimides, of which examples are the common names captan and chlorothalonil; the pyrimidines, examples of which are the common names buprimate and ethirmol; and miscellaneous compounds including acypetacs, ampropylfos, biphenyl, chloroneb, dichlofluanid, diclomezine, dichloran, dichlorphen, dipyrithione, dithianone, etridiazole, ferimzone, fluoromide, flusulfamid, formaldehyde, phthalide, nitrothal-

isopropyl, octhilinone, pencycuron, phenyl mercury acetate, 2-phenylphenol, probenazole, pyrifenox, pyroquilon, quintozene, tolyl fluanid, triazoxide, trichlamide, and triforine.

The synthetic fungicide may preferably be chosen from the group comprising anilides, alanines, carbamates, azoles, dicarboximides, phthalimides, amides and organophosphorous derivatives, more preferably from the group comprising carbamates, azoles, and phtalimides.

Said fungicide could be mancozeb, maneb, chlorothalonil, or propicinazol.

Examples of synthetic herbicides which can be combined with the saccharide preparation of the invention in combination with an enzyme include the acetamides, of which examples are the common names acetochlor and diphenamid; the amides, of which examples are the common names propyzamide and tebutam; the anilides, of which examples are the common names bromobutide and mefenacet; the aryloxylakanoic acids, of which examples are the common names dichlorprop and mecoprop; the auxins, of which examples are the common names benazolin and picloram; the bipyridyls, of which examples are the common names diquat and paraquat; the bridged diphenyls, of which examples are the common names bifenox and diclofop; the carbamates, of which examples are the common names asulam and carbetamide; the chloroacetanilides, of which examples are the common names acetochlor and pretilachlor; the dinitroanilines, of which examples are the common names butralin and trifluralin; the diphenyl ethers, of which examples are the common names furyloxyfen and lactofen; the imidazolines, of which examples are the common names imazapyran and imazethapyr; the isoxazoles, of which examples are the common names isorun and isoxabin; the isoxazolidines, of which examples are the common names clomazone and isoxapyritop; the nitriles, of which examples are the common names bromoxynil and ioxynil; the nitro compounds, of which examples are the common names chloropicrin and dinoseb; the organoarsenic derivatives, of which examples are the common names DSMA and MSMA; the organophoshorous derivatives, of which examples are the common names anilofos and butamifos; the oxidiazoles, of which examples are the common

names dimeturon and oxadiazon; the oximes, of which examples are the common names alloxydim and traloxydim; the phenoxy derivatives, of which examples are the common names fluazifop and MCPA; the phosphinic acids, of which examples are the common names glufosinate and glyphosate; the phtalic acid derivatives, of which examples are the common names chlorthal and endothal; the piperidines, of which examples are the common names dimepiperate and piperophos; the pyrazoles, of which examples are the common names benzofencap and pyrazosulfuron; the pyridiaziones, of which examples are the common names chloridazon and norflurazon; the pyridines, of which examples are the common names fluridone and triclopyr; the pyrimidines, of which examples are the common names bensulfuron and chlorimuron; the sulfonylureas, of which examples are the common names metsulfuron and tribenuronmethyl; the thiadiazoles, of which examples are the common names ethidimuron and tebuthiuron; the thiocarbamates, of which examples are the common names orbencarb and thiobencarb; the triazines, of which examples are the common names atrazine and simazine; the trifluoromethyl derivatives, of which examples are the common names diflufenican and thiazafluron; the uracils, of which examples are the common names bromacil and terbacil; the urea derivatives, of which examples are the common names bezthiazuron and isoproturon; and miscellaneous compounds, such as amitrole, blanafos, bromofenoxin, chloroacetic acid, dicamba, flamprop, flurochloridone, LS 830556, naptalam, pyridate, quinoclamine, S-23121, SMY 1500, and thifensulfuron.

The synthetic herbicides may preferably be chosen from the groups comprising phosphinic acids, phenoxy derivatives and sulfonylureas. Said herbicide could be glyphosate, fluazifop -p-butyl, and tribenuronmethyl.

Examples of synthetic nematocides which can be combined with the saccharide preparation of the invention in combination with an enzyme include the carbamates, of which examples are the common names aldicarb and carbofuran; the organochlorine derivatives, of which examples are the common names chloropicrin and DCIP; the organophosphorous derivatives, of which examples are the common names cadusafos and phorate; and miscellaneous

compounds, including dazomet, fosthiazate, methyl isothiocyanate, and oxamyl.

Examples of synthetic rodenticides which can be combined with the saccharide preparation of the invention in combination with an enzyme include the coumarins, examples of which are the common names brodifacoum and warfarin; the indandiones, of which examples are the common names diphacinone and pindone; the inorganics, examples of which are the common names hydrogen cyanide and zinc phosphide; the secosteroids, of which examples are the common names calciferol and cholecalciferol; and miscellaneous compounds including bisthiosemi, bromethalin, chloralose, chlorophacinone, difethialone, flouroacteamide, flupropadine, scilliroside, sodium fluoroacetate, and strychnine.

Examples of synthetic bactericides which can be combined with the saccharide preparation of the invention in combination with an enzyme include the antibiotics, of which examples are the common names kasugamycin and streptomycin; the quinolines, examples of which are the common names 8-hydroxyquinoline sulfate and oxolonic acid; and miscellaneous compounds including, bronopol, copper hydroxide, dichlorophen, formaldehyde, nickel dimethyldithiocarbamate, nitrapyrin, octhilinone, probenazole and tecloftalam.

Examples of synthetic molluscicides which can be combined with the saccharide preparation of the invention in combination with an enzyme include the carbamates, of which examples are the common names methiocarb and thiodicarb; and miscellaneous compounds including, fentin acetate, metaldehyde, and niclosamide.

Examples of synthetic acaricides which can be combined with the saccharide preparation of the invention in combination with an enzyme include the carbamates, of which examples are the common names aldicarb and methiocarb; the bridged dipenhyls, of which examples are the common names chlorobenzilate and dicofol; the carbamoyloximes, of which examples are the common names aldoxycarb and oxamyl; the dinintrophenyls, of which examples are the common names dinocap and DNOC; the organochlorine, of which examples are the common names dienochlor and tetradifon; the organophosphourous derivatives, of which examples are the common

names malathion and parathion; the organotins, of which examples are the common names azocyclotin and SSI-121; the phtalimides, of which examples are the common names dialifos and phosmet; the pyrimidines, of which examples are the common names diazinon and pirimiphos-methyl; and miscellaneous compounds including abamectin, AC 303,630, amitraz, bromopropylate, buprofezin, clofentezine, cufraneb, fenpyroximate, flucycloxuron, pyridaben, phosalone, rotenone, and sulfur.

The concentration of the biocide lies in the range from 0.001 to70% w/w.

The diluent or carrier in the compositions of the invention can be a solid or a liquid optionally in association with a surface-active agent, for example a dispersing agent, emulsifying agent or wetting agent. Suitable surface-active agents include anionic compounds such as a carboxylate, for example a metal carboxylate of a long chain fatty acid; an N-acylsarcosinate; mono- and di-esters of phosphoric acid with fatty alcohol ethoxylates or salts of such esters; fatty alcohol sulphates such as sodium dodecyl sulphate, sodium octadecyl sulphate or sodium cetyl sulphate; ethoxylated fatty alcohol sulphates; ethoxylated alkylphenol sulphates; lignin sulphonates; petroleum sulphonates; alkyl-aryl sulphonates such as alkyl-benzene sulphonates or lower alkylnaphthalene sulphonates, e.g. butyl-naphthalene sulphonate; salts of sulphonated naphthalene-formaldehyde condensates; salts of sulphonated phenol-formaldehyde condensates; or more complex sulphonates such as the amide sulphonates, e.g. the sulphonated condensation product of oleic acid and N-methyl taurine or the dialkyl sulphosuccinates, e.g. the sodium sulphonate of dioctyl succinate. Nonionic agents include condensation products of fatty acid esters, fatty alcohols, fatty acid amides or fatty-alkyl- or alkenyl-substituted phenols with ethylene oxide, fatty esters of polyhydric alcohol ethers, e.g. sorbitan fatty acid esters, condensation products of such esters with ethylene oxide, e.g. polyoxyethylene sorbitan fatty acid esters, block copolymers of ethylene oxide and propylene oxide, acetylenic glycols such as 2,4,7,9-tetraethyl-5-decyn-4,7-diol, or ethoxylated acetylenic glycols.

Examples of a cationic surface-active agent include, for instance, an aliphatic mono-, di-, or polyamine as an acetate, naphthenate or oleate; an oxygen-containing amine such as an amine oxide or polyoxyethylene alkylamine; an amide-linked amine prepared by the condensation of a carboxylic acid with a di- or polyamine; or a quaternary ammonium salt.

The diluent or carrier in the composition of the invention may be a solid or a liquid conventionally used for the purpose. As solid carriers bentonite diatomaceous earth, apatite, gypsum, talc, pyrophyllite, vermiculite, ground shells, and clay may be mentioned.

The composition of the invention can be in any form known in the art for the formulation of pesticides, for example, an emulsifiable concentrate, a concentrated emulsion, a multiple emulsion, an aqueous emulsion, a solution, a dispersion, a suspension concentrate, a release formulation (including a slow release formulation), a seed dressing, a granular formulation, a water soluble powder, a wettable powder, a dusting powder, a dispersible powder, an alginate, a xanthan gum and/or an aerosol. Moreover, it can be in a suitable form for direct application or as a concentrate or primary composition which requires dilution with a suitable quantity of water or other diluent before application.

An emulsifiable concentrate comprises the active ingredient and the saccharide of the invention dissolved in a water-immiscible solvent which is formed into an emulsion with water in the presence of an emulsifying agent. Another suitable concentrate is a flowable suspension concentrate which is formed by grinding the active ingredient and with water or other liquid, a wetting agent and a suspending agent.

A dusting powder comprises the active ingredient and intimately mixed and ground with a solid pulverulent diluent, for example, kaolin. A granular solid comprises the active ingredient and associated with similar diluents to those which may be employed in dusting powders, but the mixture is granulated by known methods. Alternatively it comprises the active ingredient and absorbed or adsorbed on a pre-granular diluent for example, Fuller's earth, attapulgite or limestone grit. Wettable powders,

granules or grains usually comprise the active ingredient and in admixture with a suitable surfactant and an inert powder diluent such as china clay.

The concentration of the active compounds of the invention described herein in the compositions of the invention may vary within a wide range depending on the type of formulation and the field of application.

The concentration of the saccharide in the compositions of the present invention when used in combination with a conventional biocide, as applied to pests is preferably within the range from 0.001 to 10 per cent by weight, especially 0.03 to 5 per cent by weight. In a primary composition the amount of saccharide can vary widely and can be, for example, in the range from about 5 to about 90 per cent by weight of the composition.

The preparation or the compositions of the invention with saccharide can be applied directly to the pest by, for example, spraying or dusting.

In the method of the invention the saccharide in combination with a conventional fungicide can also be applied to seeds or habitat. Thus the preparation can be applied directly to the soil before, at or after drilling so that the presence of active ingredient in the soil can control the growth of pests which may attack or influence seeds or seedlings.

When the soil is treated directly, the saccharide in admixture with the conventional biocide can be applied in any manner which allows it to be intimately mixed with the soil, such as by spraying, by broadcasting a solid form of granules, or by applying the active ingredient and at the same time as drilling by inserting it in the same drill as the seeds.

The invention is further illustrated with reference to the following examples which are not intended to be in any way limiting to the scope of the invention as claimed.

MATERIALS AND METHODS

Type of test: Prophylactic assay with foliar application.
Standard method for evaluation of fungicidal enhancement

Fungicide application

- Tomato plants are applied with 7.5 ml spraying solution each.
- Barley and wheat plants are applied with 5 ml spraying solution each
- Ventilation is on for a minimum of 3 hours after the last application. Plants remain in the application cabinet over night, with light supplied from 05.00 am to 09.00 pm.

Inoculum

Production of *Phytophtora infestans.*

Detached potato leaves are placed with the dorsal side upwards in a non-covering formation on nets in plastic containers, containing filter paper moistened with sterile water. Sporangia from infected leaves are washed with 5°C cold phosphate-calcium buffer (DS-buffer, half strength), filtered through 15 mm silk gauze, and rinsed several times with cold DS-buffer to remove bacteria. The sporangia left on the silk gauze are transferred to a clean beaker, and the concentration is determined and adjusted to $0.5 - 1.0 \times 10^5$ spores ml^{-1}.

Production of *Erysiphe graminis*

24 hours ahead of inoculation, 7-11 days old infected plants are gently rubbed to remove old asci-spores. On the day of inoculation, the same plants are shaken thoroughly to remove fresh spores. These spores are collected and weighed. 150-200 mg spores are sufficient to ensure a homogenous and effective infection level.

Production of *Puccinia recondita*

Infected leaves of wheat plants are washed with 200-500 ml sterile water with 1 drop of Triton X-155/200 ml added. The uredo spores are released by twisting and rubbing the leaves in the water. The concentration is determined and adjusted to $2.5 - 5.0 \times 10^5$ spores ml^{-1}.

Inoculation/incubation

Phytophtora infestans

Each tomato plant is sprayed with 6-8 ml spore suspension (all leaves on both dorsal and ventral sides), ensuring that the plants are covered homogeneously. The exact volume used on each plant is calculated by measuring the remaining spore suspension.

The pots are placed in sealed plexi glass boxes in trays containing 0.5 l tap water, to ensure a high level of humidity, under the following conditions: Light: 10,000 lux (minus the first 24 hours) 16 hours/day, temperature: 18°C day and 15°C night.

Erysiphe graminis

The leaves of the barley plants are fixed onto a horizontal net, ensuring a homogenous infection of all leaves, and all pots are placed in trays at the bottom of the inoculation tower. With a pressure of 0.5 bar for 30 seconds, the conidia are dispersed from the top of the tower. After 20 minutes, the exact inoculum pressure is determined by spore count on object glasses placed 5 different places in the bottom of the tower.

The pots are placed in growth compartments with the following conditions: Light: minimum 5000 lux 16 hours/day, temperature: 20°C day/night.

Puccinia recondita

The wheat plants are sprayed with 2-4 ml spore suspension, ensuring that the plants are uniformly covered. The exact volume used on each plant is calculated by measuring the remaining spore suspension.

The pots are kept dark in trays in metal containers with ½-1 liter water added, under the following conditions: Temperature: 17°C day, 15°C night. After 24 hours, the trays are moved to a growth compartment under the following conditions: Light: minimum 5000 lux 16 hours/day, temperature: 20°C day/night.

Ass ssment

Assessment is done visually by two persons, for each pathogen:

Phytophtora infestans: 4-7 days

Erysiphe graminis: 7-9 days

Puccinia recondita: 9-12 days

after inoculation, depending on disease severity (criterion - inoculated untreated plants totally infected), by the following scale:

Rank of plant infection level:

9: No infection
8: 0.1-12.5% coverage
7: 12.6-25% coverage
6: 25.1-37.5% coverage
5: 37.6-50% coverage
4: 50.1-62.5% coverage
3: 62.6-75% coverage
2: 75.1-87.5% coverage
1: 87.6-99.9% coverage
0: Whole area leaf covered (infected)

Celluclast® composition: Enzymatic proteins within the groups cellulases and hemicellulases and other proteins. Additives for stabilization are Sorbitol and salt.

EXAMPLES:

The results from the tests are expressed as mean values and ranked in SNK-groupings.

Glossary

- SNK Groupings: Mean values designated the same letter are not significantly different, mean values are separated by the Student-Newman-Keuls test.
- N is the number of plants.

- The concentration of the fungicide (F) Mancozeb is 100 ppm if nothing else is mentioned.
- The concentration of Sorbitol is 0.3 vol% pr mix. if nothing else is mentioned.
- SSP: Sorbitol, Sodium Chloride and Potassium Sorbate.

ECU

Cellulytic Activity

The cellulytic activity may be determined in endo-cellulase units (ECU) by measuring the ability of the enzyme to reduce the viscosity of a solution of carboxymethyl cellulose (CMC).

The ECU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxy-methylcellulose (CMC). The assay is carried out at 40°C; pH 7.5; 0.1M phosphate buffer; time 30 min; using a relative enzyme standard for reducing the viscosity of the CMC Hercules 7 LFD substrate; enzyme concentration approx. 0.15 ECU/ml. The arch standard is defined to 8200 ECU/g.

EGU:

Cellulytic Activity

The cellulytic activity may be measured in endo-glucanase units (EGU), determined at pH 6.0 with carboxymethyl cellulose (CMC) as substrate.

A substrate solution is prepared, containing 34.0 g/l CMC (Hercules 7 LFD) in 0.1 M phosphate buffer at pH 6.0. The enzyme sample to be analyzed is dissolved in the same buffer. 5 ml substrate solution and 0.15 ml enzyme solution are mixed and transferred to a vibration viscosimeter (e.g. MIVI 3000 from Sofraser, France), thermostated at 40°C for 30 minutes.

One EGU is defined as the amount of enzyme that reduces the viscosity to one half under these conditions. The amount of enzyme sample should be adjusted to provide 0.01-0.02 EGU/ml in the reaction mixture. The arch standard is defined as 880 EGU/g.

NCU:

Cellulytic Activity

The cellulytic activity is determined with carboxymethyl cellulose (CMC) as substrate.

One Novo Cellulase Unit (NCU) is defined as the amount of enzyme which, under standard conditions (i.e. at pH 4.80; 0.1 M acetate buffer; 10 g/l Hercules CMC type 7 LFD as substrate; an incubation temp. of 40.0°C; an incubation time of 20 min; and an enzyme concentration of approximately 0.041 NCU/ml) forms an amount of reducing carbohydrates equivalent to 1 mmol glucose per minute.

A folder AF 187.2/1 describing this analytical method in more detail is available upon request to Novo Nordisk A/S, Denmark, which folder is hereby included by reference.

FXU:

Xylanolytic Activity

The xylanolytic activity can be expressed in FXU-units, determined at pH 6.0 with remazol-xylan (4-O-methyl-D-glucurono-D-xylan dyed with Remazol Brilliant Blue R, Fluka) as substrate.

A xylanase sample is incubated with the remazol-xylan substrate. The background of non-degraded dyed substrate is precipitated by ethanol. The remaining blue colour in the supernatant (as determined spectrophotometrically at 585 nm) is proportional to the xylanase activity, and the xylanase units are then determined relatively to an enzyme standard at standard reaction conditions, i.e. at 50.0°C, pH 6.0, and 30 minutes reaction time.

A folder AF 293.6/1 describing this analytical method in more detail is available upon request to Novo Nordisk A/S, Denmark, which folder is hereby included by reference.

Example 1

Investigations were conducted, to determine whether the additives in the formulation of our enzyme-product, Celluclast®, possessed an enhancing activity on Mancozeb.

TABLE 1

Enhancing effect of 1% Celluclast® +/- autoclavation on Mancozeb (F03-05/100 ppm) against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	5.750	5	Celluclast® 10000 ppm + F
B	5.250	5	Autocl. Cell. 10000 ppm + F
C	3.125	5	F03-05/100 ppm
D	0.000	5	Inoculated

Enzyme activity:

Celluclast® 10000 ppm: 21.4 NCU/g, 0.5 FXU/g, 34.7 ECU/g, 8.2 EGU/g.

Autoclaved Celluclast® 10000 ppm: 0 NCU/g, 0 FXU/g, 0 ECU/g, 0 EGU/g.

From table 1 it can be seen that the enhancing effect of Celluclast® on the fungicide is not solely determined by the enzyme activity.

To examine which of the ingredients in the formulation provided the enhancing activity, the ingredients were examined alone and in combinations.

TABLE 2

Enhancing effect of 1% SSP, and combinations of the ingredients (Sorbitol, K-sorbate, NaCl) on Mancozeb (F03-05/100 ppm) at pH 4.5 against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	7.400	5	Sorbitol + F
C	6.600	5	Sorbitol + K-sorbate + F
D	4.600	5	Sorbitol + NaCl + F
D	4.600	5	SSP + F
E	3.600	5	NaCl + F
F	2.000	5	F03-05/100 ppm
F	2.000	5	K-sorbate + F
G	0.000	5	Inoculated.

From table 2 it can be seen that of the ingredients in the formulation of Celluclast®, sorbitol provided the biggest enhancing activity.

Example 2

With the knowledge of the effect of Sorbitol as an enhancer of the activity of Mancozeb, it was examined whether the enzyme dosage could be lowered when keeping Sorbitol level constant at a level of 1% Celluclast®.

TABLE 3

Enhancing effect of non-formulated Celluclast® (NF CEL) +/- Sorbitol (0.3 %) on Mancozeb (F03-05/100 ppm) at pH 4.5 against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	7.000	5	Celluclast® 1% +F
B C	6.800	5	NF CEL 0.5% + Sorbitol + F

SNK grouping	Mean Value	N	Treatment
B C	6.800	5	NF CEL 0.005% + Sorbitol + F
C D	6.400	5	Sorbitol + F
D	6.200	5	NF CEL 0.005% + Sorbitol + F
E	4.200	5	F03-05/100 ppm
F	2.800	5	NF CEL 0.5% + F
G	0.000	5	Inoculated

NCU activity of Celluclast® 1% = 21.4 NCU/g.
NCU activity of 0.5 % non-formulated Celluclast® = 22.0 NCU/g.

As can be seen from table 3 the same enhancing effect as Celluclast® could be obtained at low enzyme dosages by adding Sorbitol alone to a final concentration of that of 1% Celluclast®. It was found, that further enhancements with addition of non-formulated enzyme to Sorbitol were not significant. Further it was found that by far the biggest effect was provided by Sorbitol.

Example 3

Examinations were conducted as to investigate dosage relations with Sorbitol alone.

TABLE 4

Dose-response test of Sorbitol as an enhancer of Mancozeb (F03-05/100 ppm) at pH 4.5 against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	6.600	5	Sorbitol 5.0% + F
C	5.600	5	Sorbitol 1.0% + F

SNK Grouping	Mean Value	N	Treatment
C	5.600	5	Sorbitol 0.3% + F
D	4.800	5	Sorbitol 0.5% + F
D	4.600	5	Sorbitol 0.3% + F
E	3.600	5	F03-05/100 ppm
F	0.800	5	Sorbitol 5.0%
G	0.000	5	Inoculated

Table 4 shows that an increase in Sorbitol content enhanced the activity of the fungicide. At a concentration of 5%, there were no indications that this was maximum for enhancing activity.

Example 4

To examine whether Sorbitol was unique at enhancing pesticides, other polyols as well as mono- and di-saccharides were examined for enhancing activity on fungicides. The tests were performed with the saccharides at a number of moles equal to that of Sorbitol: 1.6468 mmol.

TABLE 5

Enhancing effect of Cellobiose, Fructose, N-Methylglucamine, Maltose, Sorbitol, and Xylose on Mancozeb (F03-05/100 ppm) at pH 4.5 against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	6.600	5	Fructose + F
B C	5.600	5	N-Methylglucamine + F
C D	5.600	5	Maltose + F
C D	4.800	5	Sorbitol + F

SNK Grouping	M an Value	N	Treatment
D	4.600	5	Cellobiose + F
E	3.600	5	Xylose + F
F	0.800	5	F03-05/100 ppm
G	0.000	5	Inoculated

TABLE 6

Enhancing effect of Arabinose, Glucose, Lactose, Sorbitol, Trehalose, and Xylitol on Mancozeb (F03-05/100 ppm) at pH 4.5 against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	6.200	5	Trehalose + F
C	5.400	5	Xylitol + F
C	4.400	5	Lactose + F
D	4.000	5	Sorbitol + F
D	3.800	5	Arabinose + F
E	3.000	5	Glucose + F
F	2.000	5	F03-05/100 ppm
G	0.200	5	Inoculated.

TABLE 7

Enhancing effect of Dimethyl isosorbide, Glycerol, Mannose, Meglumine diatrizoate, Sorbitol, and Sucrose on Mancozeb (F03-05/100 ppm) at pH 4.5 against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	7.400	5	Meglumine diatrizoate + F
C	6.600	5	Sorbitol + F
D	4.800	5	Sucrose + F
D	4.600	5	Glycerol + F
D	4.200	5	Mannose + F
E	2.400	5	F03-05/100 PPM
F	1.600	5	Dimethyl isosorbide + F
G	0.000	5	Inoculated

From Tables 5 to 7 it is seen that several saccharides enhanced the activity of Mancozeb.

Of major interest are the monosaccharide Fructose, the disaccharides Maltose and Trehalose, and the polyols Sorbitol and Xylitol, which all exhibit a substantial enhancing effect. Saccharides that did enhance Mancozeb, but to a lesser extent than Sorbitol, include Xylose, Cellobiose, Arabinose, Glucose, and Mannose.

Example 5

The following Tables 8 to 13 demonstrate the enhancing effect of the commercial enzyme-product, Celluclast®, which includes the saccharide Sorbitol as stabiliser, various types of fungicides and on various targets. As can be seen from the preceding Examples by far the biggest contributing factor in the enhancing activity of Celluclast® is provided by Sorbitol. Table 14 demonstrates the enhancing effect of Sorbitol on the fungicide Chlorothalonil. These date make it possible to conclude that Sorbitol (and other Saccharides) enhance the efficacy of these types of fungicides.

TABLE 8

Enhancing effect of varying concentrations of Celluclast® on Mancozeb (F03-05/100 ppm) against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	7.286	7	Celluclast® 10000 ppm + F
C	4.857	7	Celluclast® 1000 ppm + F
C	4.571	7	Celluclast® 100 ppm + F
D	3.857	7	Celluclast® 1 ppm + F
D	3.741	7	F03-05/100 ppm
E	0.000	5	Inoculated

TABLE 9

Enhancing effect of Celluclast® on Mancozeb (F03-05/100 ppm) against *Phytopthora infestans* on potato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	6.000	5	Celluclast®10000 ppm + F
C	2.200	5	F03-05/100 ppm
D	0.000	5	Inoculated

TABLE 10

Enhancing effect of varying concentrations of Celluclast® on Maneb (F23-05/140 ppm) against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Tr atment
A	9.000	6	Untreated
B	2.600	5	Celluclast® 10000 ppm + F
B C	2.200	5	Celluclast® 500 ppm + F
B C	2.000	5	Celluclast® 100 ppm + F
B C	2.000	5	Celluclast® 1000 ppm + F
C D	1.400	5	Celluclast® 1 ppm + F
C D	1.000	5	Celluclast® 10 ppm +F
D	1.000	5	F23-05/140 ppm
E	0.000	5	Inoculated

TABLE 11

Enhancing effect of varying concentrations of Celluclast® on Chlorothalonil (F19-03/20 ppm) against *Phytopthora infestans* on tomato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	2.600	5	Celluclast® 10000 ppm + F
B	2.200	5	Celluclast® 500 ppm + F
B C	2.000	5	Celluclast® 100 ppm + F
B C	2.000	5	Celluclast® 1000 ppm + F
B C	1.400	5	Celluclast® 1 ppm + F
C	1.000	5	Celluclast® 10 ppm + F
C	1.000	5	F19-03/20 ppm
D	0.000	5	Inoculated

TABLE 12

Enhancing effect of Celluclast® on Propiconazol (F02-04/1ppm) against *Erysiphe graminis* on barley plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	3.867	5	Celluclast® 30000 ppm + F
C	2.333	5	F02-04/1 ppm
D	0.400	5	Inoculated

TABLE 13

Enhancing effect of Celluclast® on Propiconazol (F0204/4 ppm) against *Puccinia recondita* on wheat plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	6.700	5	Celluclast® 30000 ppm + F
C	4.467	5	F02-04/4 ppm
D	0.733	5	Inoculated

TABLE 14

Enhancing effect of Sorbitol on Chlorothalonil (F19-03/20 ppm) against *Phytophtora infestans* on tomato plants. Applied simultaneously.

SNK Grouping	Mean Value	N	Treatment
A	9.000	6	Untreated
B	7.000	5	Sorbitol + F
C	5.400	5	F19-03/20 ppm
D	0.800	5	Inoculated

From the Tables 8 to 13 it has been demonstrated, that Celluclast® (implicit Sorbitol) is able to enhance the effect of different types of fungicides: Table 13 demonstrates the same for Sorbitol directly. The dithiocarbamates, Mancozeb (trade name Dithane DG) and Maneb (trade name Trimagol FL), and the phtalimide Chlorothalonil (trade name Daconil 500 F) (all active against *Phytophthora infestans*), as well as the conazole Propiconazole (trade name Tilt 250 EC) (active against *Erysiphe graminis*) and *Puccinia recondita*).

It has also been demonstrated that the enhancing capability is not limited to certain fungi (examples included are Oomycetes (*Phytophthora infestans*), Basidomycetes (*Puccinia recondita*), and Ascomycetes (*Erysiphe graminis*)).

Further it has been proven that the enhancing capability is not limited to certain plants (examples included are mono-cotyledons such as barley and wheat, and dicotyledons such as potato and tomato).

From the data in the tables 5 to 7 it can be seen, that the above effect also is exhibited by other saccharides.

C L A I M S

1. A composition comprising a carbohydrate in combination with a fungicidally active preparation in admixture with a biologically acceptable diluent or carrier.

2. The composition according to claim 1, wherein said carbohydrate comprises a saccharide.

3. The composition according to claim 2, wherein said saccharide comprises monosaccharides, disaccharides, polyols, polysaccharides, and salts or acids of said saccharides.

4. The composition according to claim 3, wherein said monosaccharides comprise glucose or fructose, said disaccharides comprise maltose, lactose, or trehalose, said polyols comprise sorbitol, manitol, or xylitol, said polysaccharides comprise cellulose or inulin, and said acids comprise gluconic acid.

5. The composition according to any of the claims 1 to 4, wherein the concentration of said saccharide is in the range from 0.001 to 70% w/w, especially in the range from 0.01 to 25%w/w, preferably in the range from 0.3 to 15%w/w.

6. A composition according to any of the claim 1 to 5, wherein said fungicide is chosen from the group comprising anilides, alanines, carbamates, azoles, dicarboximides, phthalimides, amides and organophosphorous derivatives, preferably from the group comprising carbamates, azoles, and phtalimides.

7. A composition according to claim 6, wherein said fungicide is mancozeb, maneb, chlorothalonil, or propicinazol.

8. The composition according to any of the claims 1 to 7, wherein the concentration of said fungicide is in the range from 0.001 to 70% w/w.

9. The composition according to any of the claims 1 to 8, further comprising an enzymatic composition, being either an enzyme, an enzyme preparation, or an enzyme complex.

10. The composition according to claim 9, wherein said enzymatic composition comprises a mixture of enzymes.

11. The composition according to any of the claims 9 and 10, wherein said enzyme preparation comprises one or more enzymes selected from the group comprising cellulases or hemicellulases.

12. The composition according to any of the claims 9 to 11, wherein said enzyme preparation comprises microbial cellulases.

13. The composition according to any of the claims 9 to 12, wherein said enzyme preparation comprises monocomponent enzymes.

14. The composition according to any of the claims 9 to 13, wherein the concentration of said enzyme is in the range from 0.001 to 5% w/w, preferably in the range from 0.01 to 2% w/w, even more preferably in the range from 0.1 to 1% w/w.

15. A method of producing a composition according to any of the claims 1 to 8, wherein said saccharide is mixed with said fungicidally active preparation.

16. A method of producing a composition according to any of the claims 9 to 14, wherein said enzymatic composition is mixed with said saccharide and said biocidally active preparation.

17. A method for controlling fungi, comprising: applying a saccharide, which is capable of enhancing the pharmacological effect of a fungicidally active preparation, in combination with said fungicidally active preparation to said fungi.

18. The method of claim 17, wherein said saccharide is applied to the fungi either prior to, simultaneously to, or subsequent to the application of said preparation.

19. The method of claim 17, wherein a composition according to any of the claims 1 to 16 is applied to said fungi.

20. The method according to any of the claims 17 to 19, wherein said fungi are plant pathogenic and storage fungi.

21. The method according to claim 20, wherein said fungi attack aerial plant parts.

22. The method according to claim 20 or 21, wherein said fungi belong to any of the classes *Ascomycetes*, *Basidomycetes*, *Myxomycetes*, *Oomycetes*, or *Deuteromycetes*.

23. The method according to any of the claims 20 to 22, wherein said fungi are *Ascomycetes* species belonging to the genera *Erysiphe*, *Basidomycetes* species belonging to

the genera *Puccinia*, or *Oomycetes* species belonging to the genera *Phytophthora*.

24. The method according to claim 23, to control *Erysiphe graminis*, *Puccinia recondita* and *Phytophthora infestans*.

25. The method according to claim 39, wherein said plants are monocotyledons or dicotyledons.

26. The method according to claim 25 to control fungi on tomato, potato, cereal crops, especially wheat and barley.

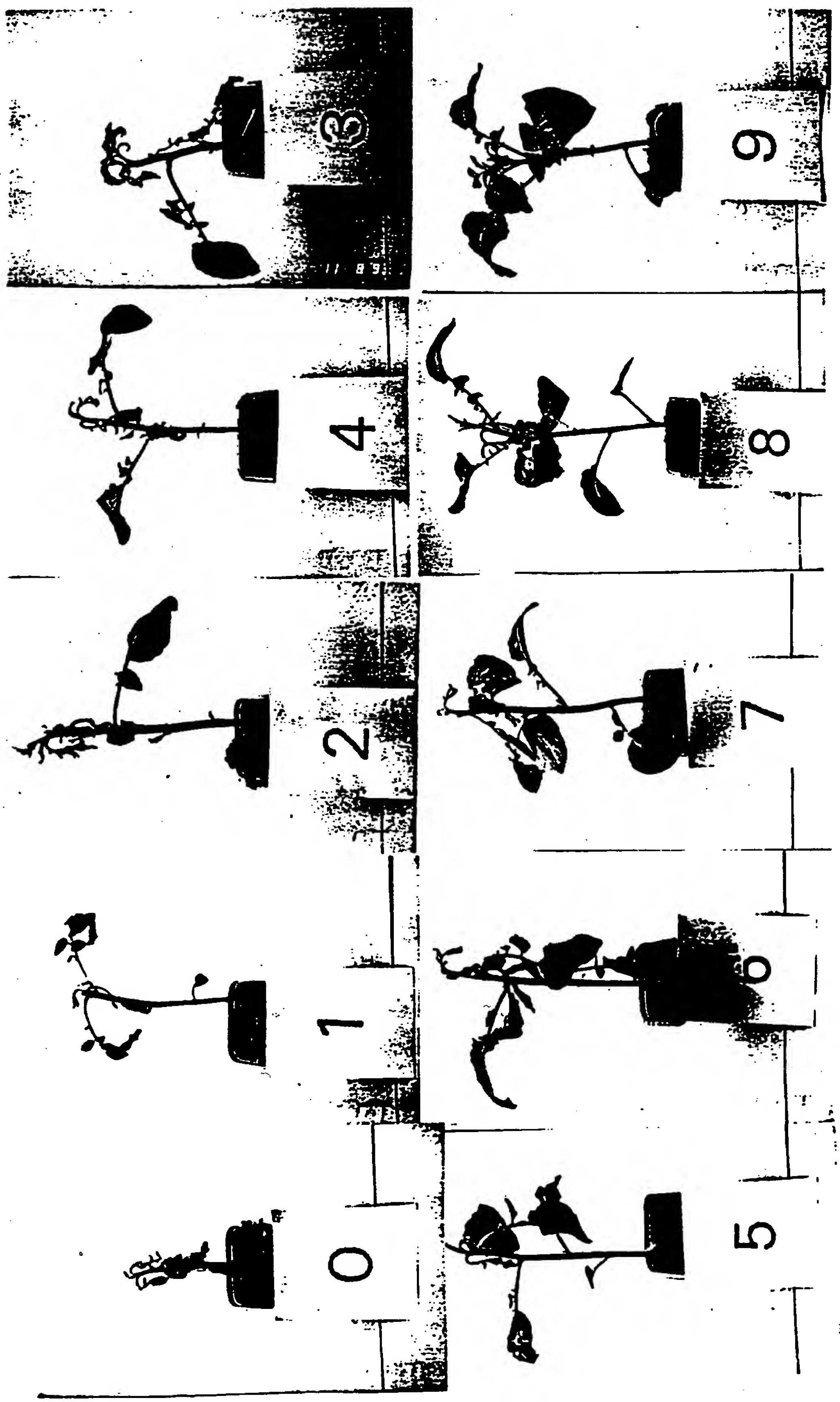

FIG. 1

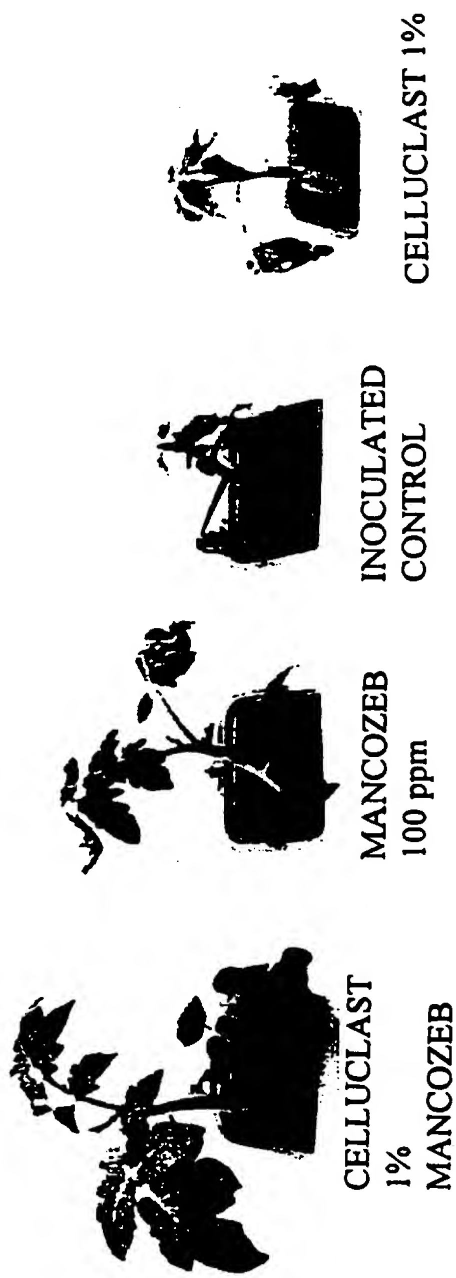
CELLUCLAST 1%
INOCULATED CONTROL
MANCOZEB 100 ppm
CELLUCLAST 1% MANCOZEB

FIG. 2